



U.S. Serial No. 09/328,130, Pending Claims After Entry of Amendment

Pursuant to 37 C.F.R. § 1.121(c)(3)

108. (Amended) A transfected primary or secondary cell having stably integrated into its genome:

- a) exogenous DNA that encodes erythropoietin, and
- b) DNA sequences that direct expression of the exogenous DNA in the primary or secondary cell.

109. (Amended) The transfected primary or secondary cell of claim 108, wherein said cell is selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

110. (Amended) The transfected primary or secondary cell of claim 108, wherein said cell is of mammalian origin.

111. (Amended) The transfected primary or secondary cell of claim 110, wherein said cell is a human cell.

112. (Amended) The transfected primary or secondary cell of claim 108, further comprising DNA encoding a selectable marker.

113. (Amended) The transfected primary or secondary cell of claim 112, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.

114. (Amended) The transfected primary or secondary cell of claim 108, wherein said cell is selected from the group consisting of:

a) a primary or secondary cell that, prior to comprising said exogenous DNA, does not make or contain erythropoietin;

b) a primary or secondary cell that, prior to comprising said exogenous DNA, makes or contains erythropoietin in less than physiologically normal amounts or in defective form; and

c) a primary or secondary cell that, prior to comprising said exogenous DNA, makes or contains erythropoietin in physiologically normal amounts.

115. (Amended) A transfected primary or secondary cell comprising:

a) exogenous nucleic acid sequences that encode erythropoietin; and

b) nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in the primary or secondary cell,

wherein the nucleic acid sequences of (a) and (b) are present in the cell episomally.

116. (Amended) The transfected primary or secondary cell of claim 115, wherein said cell is selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

117. (Amended) The transfected primary or secondary cell of claim 115, wherein said cell is of mammalian origin.

118. (Amended) The transfected primary or secondary cell of claim 117, wherein said cell is a human cell.

119. (Amended) The transfected primary or secondary cell of claim 115, further comprising nucleic acid sequences encoding a selectable marker.

120. (Amended) The transfected primary or secondary cell of claim 119, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.

121. (Amended) The transfected primary or secondary cell of claim 115, wherein said cell is selected from the group consisting of:

a) a primary or secondary cell that, prior to comprising said exogenous nucleic acid sequences, does not make or contain erythropoietin;

b) a primary or secondary cell that, prior to comprising said exogenous nucleic acid sequences, makes or contains erythropoietin in less than physiologically normal amounts or in defective form; and

c) a primary or secondary cell that, prior to comprising said exogenous nucleic acid sequences, makes or contains erythropoietin in physiologically normal amounts.

122. (Amended) A clonal cell strain of transfected secondary cells that express exogenous nucleic acid sequences encoding erythropoietin present therein.

123. (Amended) The clonal cell strain of claim 122, wherein the exogenous nucleic acid sequences are stably incorporated into genomic DNA of the transfected secondary cells.

124. (Amended) The clonal cell strain of claim 122, wherein said transfected secondary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

125. (Amended) The clonal cell strain of claim 122, wherein said transfected secondary cells are of mammalian origin.

126. (Amended) The clonal cell strain of claim 125, wherein said transfected secondary cells are human cells.

127. (Amended) The clonal cell strain of claim 122, wherein the exogenous nucleic acid sequences are present in the transfected secondary cells episomally.

128. (Amended) A heterogenous cell strain of transfected secondary cells having stably incorporated into their genomes:

a) exogenous DNA encoding erythropoietin, and

b) DNA sequences that direct expression of the exogenous DNA in the secondary cells.

129. (Amended) The heterogenous cell strain of claim 128, wherein the transfected secondary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

130. (Amended) The heterogenous cell strain of claim 128, wherein said transfected secondary cells are of mammalian origin.

131. (Amended) The heterogenous cell strain of claim 130, wherein said transfected secondary cells are human cells.

132. (Amended) A mixture of cells consisting essentially of transfected primary or secondary cells of claim 108 and primary or secondary cells that do not comprise said exogenous DNA.

133. (Amended) A method of producing a clonal cell strain of transfected secondary cells that express exogenous nucleic acid sequences encoding erythropoietin, said method comprising the steps of:

a) providing a mixture of cells comprising primary cells;

b) transfecting into primary cells provided in (a) a nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin and nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in the primary cells, thereby producing transfected primary cells that express the exogenous nucleic acid sequences encoding erythropoietin; and

c) culturing a transfected primary cell produced in (b) to produce a clonal cell strain of transfected secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin.

136. The method of claim 133, wherein said primary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

137. The method of claim 133, wherein said primary cells are of mammalian origin.

138. The method of claim 137, wherein said primary cells are human cells.

139. (Amended) The method of claim 133, wherein, in step (b), nucleic acid sequences encoding a selectable marker are transfected into primary cells provided in (a).

140. The method of claim 139, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.

141. (Amended) The method of claim 133, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into primary cells provided in (a) by electroporation

to produce at least one primary cell having the exogenous nucleic acid sequences stably integrated into genomic DNA.

142. The method of claim 141, wherein electroporation is carried out at an electroporation voltage of between 250 and 300 volts and a capacitance setting of approximately 960 μ Farads.

143. (Amended) The method of claim 133, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into primary cells provided in (a) by microinjection.

144. (Amended) The method of claim 133, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into primary cells provided in (a) by a transfection method selected from the group consisting of calcium phosphate precipitation, modified calcium phosphate precipitation, liposome fusion methodologies, receptor mediated transfer, micro-projectile bombardment, and polybrene precipitation.

145. The method of claim 133, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into genomic DNA by homologous recombination

between DNA sequences present in the nucleic acid molecule construct and genomic DNA.

146. (Amended) A method of producing a clonal cell strain of transfected secondary cells that express exogenous nucleic acid sequences encoding erythropoietin, said method comprising the steps of:

- a) providing a mixture of cells comprising primary cells;
- b) producing a population of secondary cells from primary cells provided in (a);
- c) transfecting into secondary cells produced in (b) a nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin and nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in the secondary cells, thereby producing transfected secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin; and
- d) culturing a transfected secondary cell produced in (c) to produce a clonal cell strain of transfected secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin.

149. The method of claim 146, wherein said primary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

150. The method of claim 146, wherein said primary cells are of mammalian origin.

151. The method of claim 150, wherein said primary cells are human cells.

152. (Amended) The method of claim 146, wherein, in step (c), nucleic acid sequences encoding a selectable marker are transfected into secondary cells produced in (b).

153. The method of claim 152, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.

154. (Amended) The method of claim 146, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into secondary cells produced in (b) by electroporation to produce at least one secondary cell having the exogenous nucleic acid sequences stably integrated into genomic DNA.

155. The method of claim 154, wherein electroporation is carried out at an electroporation voltage of between 250 and 300 volts and a capacitance setting of approximately 960 μ Farads.

156. (Amended) The method of claim 146, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into secondary cells produced in (b) by microinjection.

157. (Amended) The method of claim 146, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into secondary cells produced in (b) by a transfection method selected from the group consisting of calcium phosphate precipitation, modified calcium phosphate precipitation, liposome fusion methodologies, receptor mediated transfer, micro-projectile bombardment, and polybrene precipitation.

158. The method of claim 146, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into genomic DNA by homologous recombination between DNA sequences present in the nucleic acid molecule construct and genomic DNA.

159. (Amended) A method of producing a heterogenous cell strain of transfected secondary cells that express exogenous nucleic acid sequences encoding erythropoietin, said method comprising the steps of:

a) providing a mixture of cells comprising primary cells;

b) transfecting into primary cells provided in (a) a nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin and nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in the primary cells, thereby producing a mixture of primary cells that includes transfected primary cells that express the exogenous nucleic acid sequences encoding erythropoietin;

c) culturing the product of (b) to produce a heterogenous cell strain of transfected secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin.

162. The method of claim 159, wherein said primary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

163. The method of claim 159, wherein said primary cells are of mammalian origin.

164. The method of claim 163, wherein said primary cells are human cells.

165. (Amended) The method of claim 159, wherein, in step (b), nucleic acid sequences encoding a selectable marker are transfected into primary cells provided in (a).

166. The method of claim 165, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.

167. (Amended) The method of claim 159, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into primary cells provided in (a) by electroporation to produce at least one primary cell having the exogenous nucleic acid sequences stably integrated into genomic DNA.

168. The method of claim 167, wherein electroporation is carried out at an electroporation voltage of between 250 and 300 volts and a capacitance setting of approximately 960 μ Farads.

169. (Amended) The method of claim 159, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into primary cells provided in (a) by microinjection.

170. (Amended) The method of claim 159, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into primary cells provided in (a) by a transfection method selected from the group consisting of calcium phosphate precipitation, modified calcium phosphate precipitation, liposome fusion methodologies, receptor mediated transfer, micro-projectile bombardment, and polybrene precipitation.

171. The method of claim 159, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into genomic DNA by homologous recombination between nucleic acid sequences present in the nucleic acid molecule construct and genomic DNA.

172. (Amended) A method of producing a heterogenous cell strain of transfected secondary cells that express exogenous nucleic acid sequences encoding erythropoietin, said method comprising the steps of:

a) providing a mixture of cells comprising primary cells;

b) producing a population of secondary cells from primary cells provided in (a);

c) transfecting into secondary cells produced in (b) a nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin and nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in secondary cells, thereby producing a mixture of secondary cells that includes transfected secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin;

d) culturing the product of (c) to produce a heterogenous cell strain of transfected secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin.

175. The method of claim 172, wherein said primary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

176. The method of claim 172, wherein said primary cells are of mammalian origin.

177. The method of claim 172, wherein said primary cells are human cells.

178. (Amended) The method of claim 172, wherein, in step (c), nucleic acid sequences encoding a selectable marker are transfected into secondary cells produced in (b).

179. The method of claim 178, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.

180. (Amended) The method of claim 172, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into secondary cells produced in (b) by electroporation to produce at least one secondary cell having the exogenous nucleic acid sequences stably integrated into genomic DNA.

181. The method of claim 180, wherein electroporation is carried out at an electroporation voltage of between 250 and 300 volts and a capacitance setting of approximately 960 μ Farads.

182. (Amended) The method of claim 172, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into secondary cells produced in (b) by

microinjection.

183. (Amended) The method of claim 172, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is transfected into secondary cells produced in (b) by a method selected from the group consisting of calcium phosphate precipitation, modified calcium phosphate precipitation, liposome fusion methodologies, receptor mediated transfer, micro-projectile bombardment, and polybrene precipitation.

184. The method of claim 172, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into genomic DNA by homologous recombination between DNA sequences present in the nucleic acid molecule construct and genomic DNA.